Evaluation of two novel plant gums for bioadhesive microsphere and sustained-release formulations of metformin hydrochloride

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Abstract

Background. The biological half life of metformin requires multiple doses which are associated with poor patient compliance. This justifies the need for a dosage form with reduced dosing frequency.

Objectives. Gums from *Enterolobium cyclocarpum* and *Cedrela odorata* trees were evaluated in formulating bioadhesive microspheres containing metformin hydrochloride, for sustained drug release. Hydroxylpropylmethyl cellulose (HPMC) was the standard.

Material and methods. Microspheres were produced from formulations of API and either cedrela gum (FC), enterolobium gum (FE) or HPMC (FH), using a W/O solvent extraction technique. The microspheres were characterized using a particle size analyzer, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffractometer (PXRD), drug entrapment, in vitro release and mucoadhesion studies. The data was analyzed using ANOVA and t-test at $p = 0.05$.

Results. FT-IR spectroscopy indicated no alteration in the functional groups of metformin. A yield of 92–98% microspheres was obtained from all the formulations which had a particle size range of 72–84 µm. SEM revealed cylindrical to near-spherical particles with rough surfaces. The drug release profile showed a burst over the first 30 min followed by a steady release for about 5 h and a slow release for 5 days. Formulations containing the gums sustained the release of API for almost the same time as HPMC formulations; the ranking order was FE > FH > FC ($p > 0.05$). All the formulations exhibited good concentration-dependent mucoadhesive properties.

Conclusions. The gums were suitable for formulation of mucoadhesive microspheres for sustained release of metformin. The formulations showed good release properties in an alkaline pH.

Key words: microspheres, metformin, bioadhesion, enterolobium gum, cedrela gum

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Microspheres are small, spherical particles with a diameter in the micrometer range (1–1000 μm), obtainable from synthetic and natural materials, suitable for drug loading and release. Drug delivery systems that couple mucoadhesive properties to microspheres would be capable of enhancing intimate contact, better bioavailability, specific drug targeting and sustained release properties.

The potentially toxic effects of synthetic polymers is a major drawback in their use as drug carriers. Hence, the use of natural polymers that are biodegradable, cheap and easily accessible is becoming important in the design of drug delivery systems. Bioadhesion is a process by which macro-molecules stick to the mucosal surfaces in the body and remain there for a reasonable length of time. When these materials are loaded with active pharmaceutical ingredients (API), they enhance the release of the drug substance for either local or systemic absorption. A more specific term is mucoadhesion; adhesion of materials to surfaces of the body such as the nose and mouth that are covered by mucin molecules.[1] Mucoadhesion has been defined as the interfacial force interaction between polymeric materials and mucosal tissues.[2] Mucoadhesive agents are usually polymers and they contain hydrogen bonds, useful in wet formulations or in dry powders for drug delivery purposes. For mucoadhesion to occur, there must be close contact between the mucoadhesive agent and the mucus, followed by the formation of chemical bonds between the macro-molecules.

Plant gums are polysaccharides of natural origin. They are adhesive in nature and produced as exudates from the incised bark of trees and shrubs. Plant gums are generally hydrophilic with some being soluble in water while others produce mucilages by absorbing water. Gums have been found to be useful in the food, pharmaceutical, paper and textile industries.[3]

The *Cedrela odorata* tree, from which cedrela gum is obtained, is an important tree species in the family chinaberry, *Meliaceae*. It is commonly called Spanish cedar or Cuban cedar. It is a tree in the new world tropics appearing in the forests of moist and seasonally dry subtropical or tropical life zones. Its primary use is in storing household clothing. It contains aromatic and insect repelling resins and is often used in honey production. The polysaccharides from the gum contain galactose, arabinose and rhamnose as neutral sugars and uronic acids as residues. The cationic components of the ash are mainly calcium and magnesium. Cedrela gum has a non-Newtonian flow behavior characterized by lack of a low-shear limiting Newtonian viscosity plateau even at low shear rates. The average flow index value is low but infinite shear rate viscosity is high.[4] Previous works showed cedrela gum to have mucoadhesive properties.[5]

Enterolobium gum, produced by the *Enterolobium cyclocarpus* tree family *Mimosoideae* of the *Leguminosae*, is commonly known as the guanacaste or elephant-ear tree. It is a species of flowering tree, native to Central America and now widely distributed throughout the tropics where it is planted mainly as a roadside or garden tree.[6] The tree is useful for its production of highly palatable and nutritious pods, containing sugary dry pulp. The pods are also used as food when cooked as a vegetable. Extracts from the bark have medicinal properties and have been used against colds and bronchitis.[7] A structural study of the gum using chemical methods and NMR spectroscopy showed the structure is essentially a β-(1-3)-galactan, with the presence of α-L-arabinopyranose.[8] The gum contains galactose, arabinose, rhamnose and glucuronic acid as the main monosaccharides, it has a high concentration of uronic acid and is highly viscous in nature.[9] The use of enterolobium gum in pharmaceutical dosage forms has not yet been investigated.

The use of gums as polymers in pharmaceutical formulations has been reported; Akpabio et al.[10] formulated and evaluated sustained release tablets produced from *Lesianthera africana* gum, Adedokun et al.[11] studied the compressional, mechanical and release properties of *Eucalyptus tereticornis* in paracetamol tablet formulations, Emeje et al.[12] worked on the formulation properties of *Cissus refescence* gum, while Odeniyi et al.[2] reported the release and mucoadhesive properties of diclofenac matrix tablets from natural and synthetic polymer blends. In the present study, we have investigated extracts from 2 native trees, whose use in drug formulation is not yet reported.

Hydroxy propyl methylcellulose (HPMC) was used as a standard polymer. It has a reversible thermal gelation property and forms hydrophilic matrices which mainly act by means of diffusion in controlling drug release. HPMC was used in this study due to its reported significant adhesive properties in tablet dosage forms.[5,13]

Natural mucoadhesive substances considerably swell in water and form a gelatinous mass.[14] This gelling and the mucoadhesive properties of cedrela gum have been reported.[2] The similarity in the polysaccharide composition of cedrela and enterolobium gums suggests similar mucoadhesive and gelling properties, hence the basis for comparison of these 2 natural polymers with HPMC as the standard. Formulating mucoadhesive microspheres from these natural polymers is expected to offer the possibility of intimate contact between a drug delivery system and mucous membranes, and sustained release of the loaded API.

Metformin is the first-line drug in the management of Type II diabetes. Metformin is believed to be the most widely orally used medication for diabetes and it is also used in polycystic ovary syndrome.[15] Metformin has bioavailability of 50–60% under fasting conditions, it reaches peak plasma concentration within 1–3 h of administration of immediate-release and 4–8 h with extended-release formulations, with an average elimination half life of 6.2 h.[16] The short elimination half life is a limitation which necessitates frequent administration, 2–3 times daily, leading to poor patient compliance and adherence.

The high release profile of metformin from pectin microspheres has been reported.[17] Also, the dissolution rate of metformin hydrochloride in phosphate buffer (pH 6.8) was studied in different formulations and was found to be as high as between 96.27 and 97.93%.[18]

This paper evaluates the solid-state characteristics and physicochemical properties of 2 novel plant gums (enterolobium and cedrela gums) in the formulation of bioadhesive microspheres loaded with metformin hydrochloride with the target of reducing the dosing frequency of metformin through a sustained-release drug delivery system.

Material and methods

Material

The materials used in this work include enterolobium gum (ET), obtained from the *Enterolobium cyclocarpus (Mimosoideae)* tree, cedrela gum (CD), obtained from the *Cedrela odorata (Meliaceae)* tree, and hydroxy propyl methylcellulose (HPMC) from Colorcon Asa Limited India. Metformin hydrochloride, from Arbro Pharmaceuticals Limited, India was the model drug and the reagents were of AR grade.

Methods

Extraction of the gum

The cedrela and enterolobium gums were collected from *Cedrela odorata* and *Enterolobium cyclocarpus* trees, respectively, and authenticated at the Botany Department, University of Ibadan, Nigeria. The collected gum was purified using the established procedure and then hydrated by soaking in a chloroform/water mixture of 0.5/95.5% V/V for 5 days, while stirring from time to time.[19] Unwanted materials were removed by straining the gum through a muslin cloth. The gum was precipitated from the solution by absolute ethanol, filtered and washed with diethyl ether and then dried in the oven at 40°C for 18 h to ensure complete removal of associated earth particle and toxic residues.[20–22] The gum was milled in a domestic blender and sieved. Materials of particle size of < 200 µm were collected and used for all investigations.

Fourier Transform Infrared (FT-IR) spectroscopy

The possibility of interaction between the pure drug (metformin) and each of the polymers in the final formulations was established by recording their spectra on the FT-IR spectroscope (Model 2000 Perkin Elmer Spectroscopy, USA). Samples were prepared in KBr discs (1% w/w). A scanning range of 1000–4500 cm^{-1} was used.

Preparation of samples for formulation

Material blends for microsphere formulations were made in their various proportions (Table 1), containing metformin and polymer in ratios 1 : 1, 1 : 2, 1 : 3 and 1 : 4. The component powders were mixed in a planetary mixer for 5 min to ensure homogeneity. Formulations were stored in air-tight containers.

Formulation of microspheres

Metformin microsphere beads were formulated by the W/O emulsion solvent evaporation technique.[17] Different drug : polymer ratios were used (Table 1). The drug (500 mg) and the gum (500, 1000, 1500 or 2000 mg) were dispersed in water. The slurry formed was transferred into 200 mL of liquid paraffin and 0.5% Span 80 was added as the emulsifying agent. The system was emulsified by stirring in a 500 mL beaker at a temperature of 80°C and 200 rpm on a magnetic stirrer for 2.5 h. On evaporation of the aqueous phase, the oil was decanted to collect the microspheres formed. Filtration was carried out using no. 1 Whatman filter paper and the microspheres were washed repeatedly with n-hexane to remove the oil. The microspheres were dried in an oven at 60°C for 2 h and then stored in a desiccator over fused calcium chloride.

Evaluation of the microspheres

1. Drug content

A quantity of 100 mg microspheres was taken from each formulation and powdered using a mortar and pestle. The powder was suspended in methanolic water to form a 1 in 100 mL suspension. The suspension was agitated and then filtered through a 0.45 µm membrane filter. Metformin content was determined spectrophoto-

Table 1. Thermal behavior of polymers

Material	Onset temperature, T_0 (°C)	Endset temperature, T_e (°C)	Peak, T_p (°C)	Enthalpy change, ΔH (J/g)
Cedrela gum	57.40	112.73	76.41	513.40
Enterolobium gum	50.06	139.98	77.47	705.31
HPMC	52.55	115.61	80.45	520.62
Metformin	221.45	225.65	222.45	450.55
FC 3	85.22	118.23	70.32	495.20
FE 3	62.45	122.55	72.12	500.52
FH 3	75.22	120.22	75.35	477.34

metrically at 233 nm using a regression equation from the standard calibration curve.

2. Percent microsphere yield

The yield was calculated as the weight of the microspheres recovered from each batch divided by the total weight of the API and polymer multiplied by 100.

3. Entrapment efficiency

A quantity (100 mg) of the drug-loaded microspheres was dispersed in 100 mL of methanolic water. The resultant dispersion was agitated and filtered through a 0.45 μm membrane filter. Drug content was determined spectrophotometrically at 233 nm, using a regression equation from a standard graph. Entrapment efficiency was calculated as follows:

$$EE = PC/TC \times 100 \quad (1),$$

where PC is the practical drug content and TC is the theoretical drug content. Determinations were done in triplicate.

4. Particle shape and morphology

The shape and surface topography of the microspheres were studied using a scanning electron microscope (Hitachi Japan, Model S3400N). Gold coating was used to make the samples electrically conductive.

5. Moisture content

The moisture content of the prepared microspheres was determined on a Moisture Balance (Mettler PM480 Delta Range). Determinations were done in triplicate.

6. Particle size and size distribution of microspheres

The particle size and size distribution of the polymers and microsphere formulations were determined by microscopy method. Samples of the microspheres were dispersed in normal saline containing 0.1% Tween 80 and photographed under a light microscope on which an ocular micrometer and a light camera are mounted (MT3300EXII, Microtrac-Bel, Japan). Hundred mL normal saline solution containing 0.1 mL Tween 80 was used to prepare the samples of microspheres to be mounted on the microscope. Approximately 1–2 drops of the solution was placed on the microscope slide and 100 mg of microspheres were dispersed carefully in the solution. Approximately 200 microspheres were counted and the mean diameter determined.[23]

7. Powder X-ray diffraction (PXRD) study

Powder samples of the plain API, polymers and metformin-loaded microspheres were subjected to PXRD studies on an X-ray diffractometer (Rigaku Miniflex 600, Japan). The following conditions were used: a slit-detector Cu Kα radiation source (30 kV, 15 mA, $\lambda = 0.15418$ nm), 2θ scan range was 3–35° and a scan rate of 4°/min under ambient temperature. This was carried out to detect any changes in the crystallinity of the API in the microsphere formulations.

8. Differential scanning calorimetry (DSC)

To further investigate the presence of any interaction between the polymers and the API, the thermal transition of the plain API, polymers and drug-loaded microspheres were assessed by DSC (PerkinElmer, USA apparatus). The DSC was calibrated using indium as a reference standard (5 mg, 99.999% pure, onset at 156.6°C) and then the thermal behavior of the samples was measured. Approximately 5 mg of each sample was placed in a sealed aluminum pan and heated from 25 to 230°C at a scanning rate of 10°C/min under a nitrogen flow of 20 mL/min.

9. In vitro drug release

The drug release profile from the microspheres was measured using a Dissolution Tester (USP ELECTROLAB TDT-08L). A volume of 900 mL of 6.8 pH phosphate buffer was used as the dissolution medium. A bath temperature of 37±2°C and basket rotation of 100 rpm was maintained throughout the period of measurement.[24] A microsphere formulation equivalent of 100 mg metformin hydrochloride was used. Samples (5 mL) were withdrawn at time 0, 5, 10, 15, 30 and 60 min and then at 1 h intervals for 9 h and at 24 h intervals for five days. Each withdrawal was replaced by a fresh 5 mL phosphate buffer solution. The samples withdrawn were filtered through a 0.45 μm membrane filter and then the drug content in each withdrawn sample was determined on a UV-Visible Spectrophotometer (SPECORD 200 Analyticjena) at 233 nm. Determinations were done in triplicate.

10. Mechanism of drug release from microspheres

The mechanism of metformin release was determined by analyzing the drug release data with the zero order kinetic, first order kinetic, Higuchi model, Hixon-Crowell and Korsemeyer-Peppas equations. The constants of release kinetic and coefficient of correlation (r^2) were obtained from slopes of plots by linear regression analysis. However, in order to determine the mechanism of drug release, the release data was fitted in a Korsemeyer-Peppas equation:[21,2]

$$\text{Log } (Mt/Mf) = \text{Log } k + n\text{Log } t \quad (2).$$

This equation describes drug release behavior from polymeric systems. Mt is the amount of drug release at time t, Mf is the amount of drug release after infinite time; k is a release rate constant incorporating the structural and geometric characteristics of the dosage form and n is the diffusional exponent, which indicates the mechanism of drug release. For a cylinder shaped matrix, the value of $n = 0.45$ indicates Fickian (case I) release; > 0.45 but < 0.89 for non-Fickian (anomalous) release; and > 0 indicates a super case II type of release. The case II mechanism refers to the erosion of the polymer and anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled drug release. The mean dissolution time (MDT) is a more accurate drug release rate than the tx%. The equation is used to characterize drug release rate from the dosage form and the retarding efficiency of the polymer. Values of MDT can be calculated from dissolution data using the equation:

$$MDT = (n/n + 1)k - 1/n \quad (3),$$

where n is the release exponent and k is release rate constant. A higher value of MDT indicates a higher drug retaining ability of the polymer.[2]

11. Mucoadhesive properties

The mucoadhesive properties of the formulations were assessed ex vivo according to the method used by Odeniyi et al.[2] An ileum segment of a butchered goat, freshly incised, was obtained from the slaughterhouse, Faculty of Agriculture, University of Ibadan, Nigeria. Approximately 100 mg of each microsphere formulation was attached to the base of an aluminum probe, fixed to the mobile arm of a Texture Analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK). The attached sample was lowered slowly at a rate of 0.1 mm/s to make contact with the ileum. A contact force of 0.25 N between the ileum and the microsphere was maintained for 5 min. The aluminum probe was withdrawn at the slow rate of 0.1 mm/s. The force required to detach the microsphere from the intestine was recorded as a measure of the bioadhesion. Determinations were done in triplicate.

12. Statistical analysis

The results obtained were subjected to statistical analysis using ANOVA, followed by posthoc Tukey's test, where more than two sets of data were obtained, to determine the level of significance (p-value) of an effect or the difference between means. Parameters that are significant at 95% confidence were considered significant or different at p = 0.05.

Discussion

FT-IR spectroscopy

Possible drug-polymer interaction was studied by FT-IR spectroscopy; the infrared spectra are shown in Fig. 1a–e. There was no difference in the bands shown by the plain drug and when formulated with each of the polymers. This indicates that the functional groups were not altered in the formulations due to interaction between metformin hydrochloride and the polymers. However, reduction in the intensities of the bands of metformin was observed in all the formulations; this is due to the reduction in crystallinity of the API by the amorphous polymers.

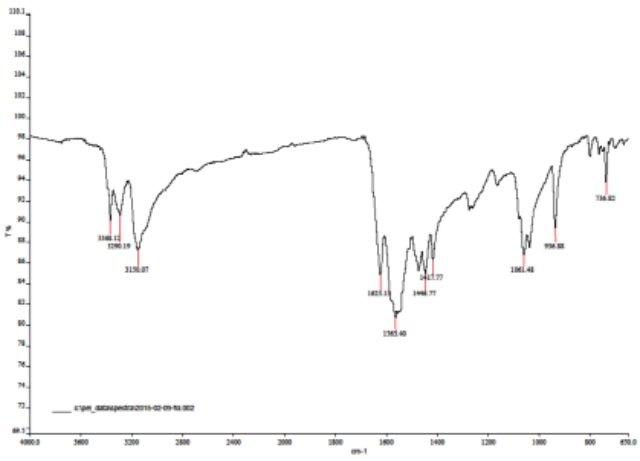

Fig. 1a. FT-IR of metformin

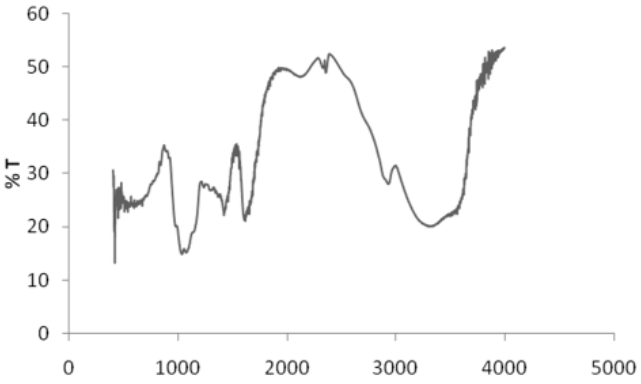

Fig. 1b. FT-IR of ET

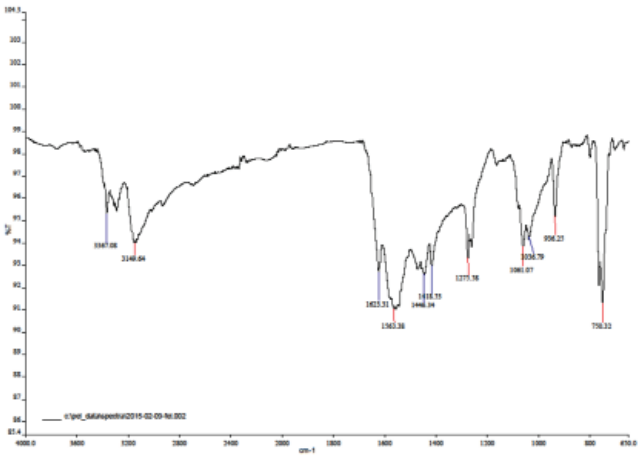

Fig. 1c. FT-IR spectra of FE

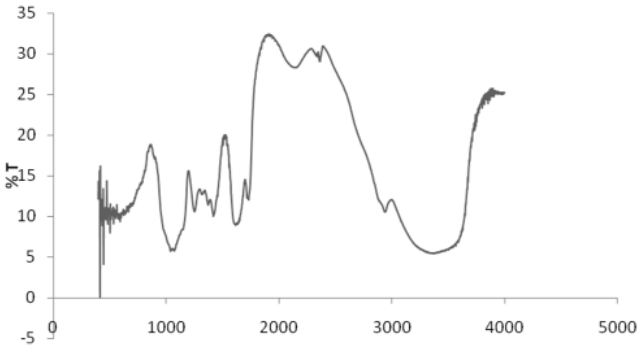

Fig. 1d. FT-IR of CD

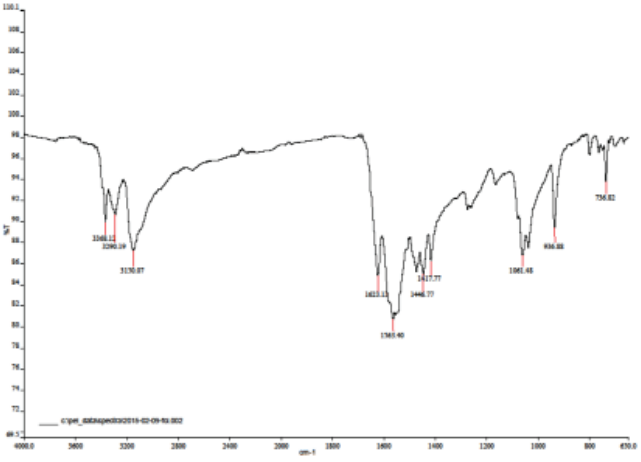

Fig. 1e. FT-IR of FC

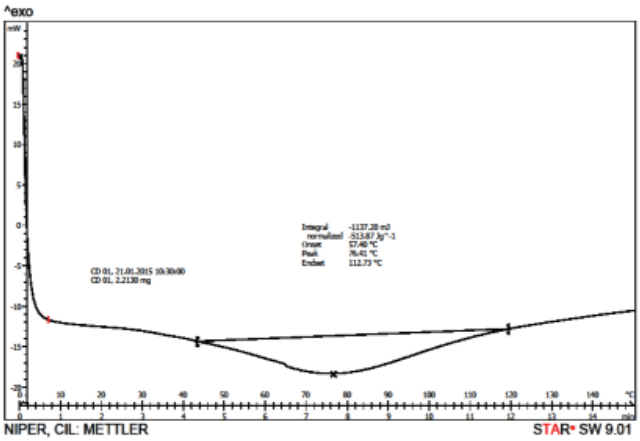

Fig. 2a. Differential scanning calorimetry of cedrela gum

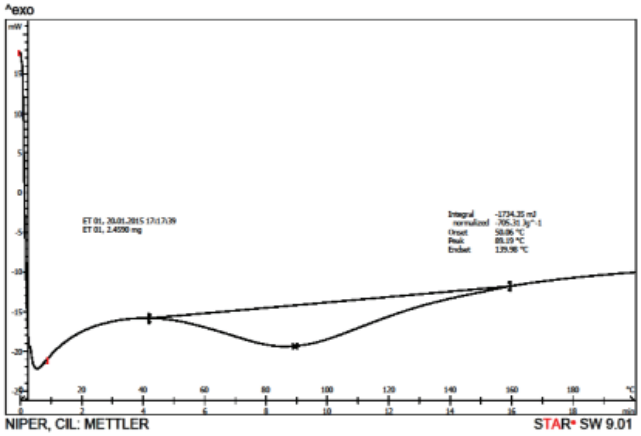

Fig. 2b. Differential scanning calorimetry of enterolobium gum

Drug-polymer interaction

To further study API-polymer interaction, the behavior of the individual polymers and microsphere formulations was studied by Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction (PXRD).

DSC is an analytical technique used to determine the quantity of heat either absorbed or released when a material undergoes physical or chemical changes.[26] Fig. 2a–b shows the thermal behavior of cedrela and enterolobium gums.

There was a wide range of temperatures between the onset and endset temperatures for the 2 polymers (Table 1); this indicates the amorphous nature of the gums. There was a sharp endothermic peak observed for metformin at 222.452°C, indicating the presence of a crystalline drug and the relative purity of the metformin drug sample compared to the polymers. Lower peaks were obtained for the polymers; this showed the amorphous nature of the gums and HPMC. The relatively low values obtained for the enthalpy change is attributable to the absence of the long chain of amylopectin molecules as found in starches.[27] Furthermore, the microsphere formulations generally reduced the intensity of bands of metformin; this is due to the reduction of crystallinity of the API when being loaded into the polymers.

The PXRD studies (Fig. 3a and 3b) show the diffractograms of metformin, the polymers and the microsphere formulations. The polymers generally showed the broad peaks of a halo pattern which indicates an amorphous nature. The diffraction patterns of amorphous solids consist of broad peaks often referred to as an amorphous halo because amorphous systems have little long-range order.[28] Metformin displayed crystallinity by showing peaks at 2θ of 12, 13, 18, 22, 24, 25, 32, 34. Similar peaks were produced by the microsphere formulations but with significantly reduced intensity. This can be attributed to

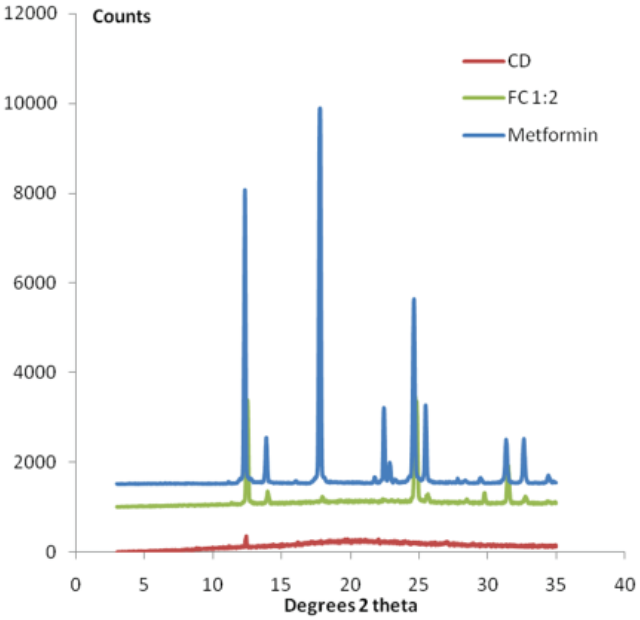

Fig. 3a. PXRD diffractograms of metformin, cedrela gum and FC at ratio 1 : 2

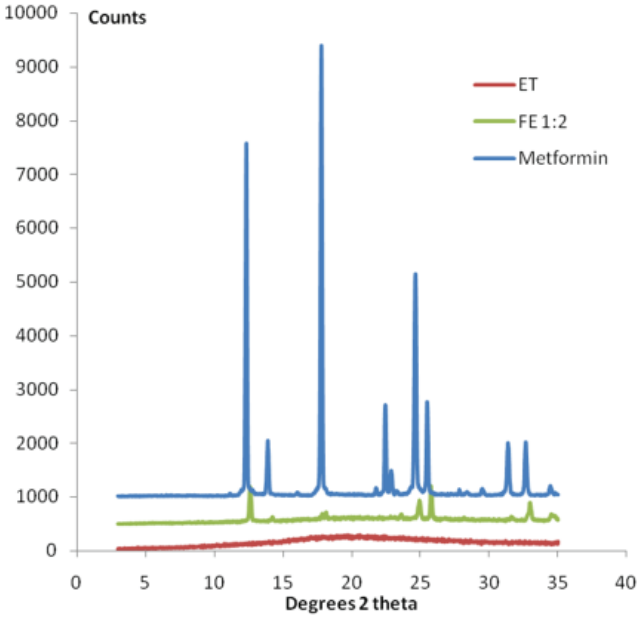

Fig. 3b. PXRD diffractograms of metformin, enterolobium gum and FE at ratio 1 : 2

Table 2. Properties of the microspheres

Formulations	Yield (%)	Actual drug content (mg)	Theoretical drug content (mg)	Drug entrapment efficiency (%)	Mean particle size (µm)	Moisture content (%)	Bioadhesion (peak detachment force) (N)
FC1	92.00 ±12.23	380.00 ±2.3	500	76.01 ±2.11	80.10 ±0.02	8.21 ±0.15	1.22 ±0.12
FC 2	96.70 ±15.12	387.40 ±2.5	500	77.48 ±1.45	84.20 ±0.34	8.78 ±2.1	1.22 ±0.23
FC 3	97.70 ±15.11	390.10 ±2.1	500	78.02 ±0.25	81.40 ±0.25	10.24 ±5.21	1.25 ±0.15
FC 4	94.40 ±8.25	400.05 ±2.0	500	80.01 ±5.45	81.70 ±0.22	10.20 ±2.35	1.27 ±0.14
FE1	96.80 ±10.11	380.50 ±1.6	500	76.10 ±4.45	75.60 ±0.15	8.96 ±3.01	1.32 ±1.45
FE2	92.50 ±22.12	387.05 ±1.8	500	77.41 ±7.10	77.60 ±0.17	6.80 ±5.01	1.33 ±0.91
FE 3	98.10 ±10.50	392.50 ±1.6	500	78.50 ±1.55	77.00 ±0.11	8.85 ±11.50	1.38 ±0.15
FE 4	98.90 ±8.75	403.07 ±1.5	500	80.61 ±4.72	72.00 ±0.13	9.65 ±7.11	1.38 ±0.15
FH 1	94.60 ±9.45	381.15 ±22	500	76.23 ±0.35	78.80 ±0.22	4.00 ±0.01	1.31 ±1.55
FH 2	95.10 ±12.15	386.25 ±2.2	500	77.25 ±3.75	77.80 ±1.25	3.880 ±6.01	1.31 ±0.55
FH 3	97.50 ±10.11	397.45 ±3.1	500	79.49 ±2.28	77.50 ±0.45	3.98 ±4.01	1.33 ±0.55
FH 4	98.40 ±8.55	404.15 ±2.0	500	80.83 ±2.69	77.80 ±0.22	4.75 ±7.55	1.35 ±0.15

a dilution effect or decrease in crystallinity of the API after incorporation into microspheres. This is expected to be an advantage in the delivery of metformin from the dosage form because the conversion from the crystalline form of metformin to the amorphous form will enhance better dissolution.

Evaluation of the microspheres

Microspheres loaded with metformin were prepared by the W/O emulsion solvent evaporation technique. The polymers formed mucilaginous dispersion in water with good swelling properties. The percent yield of microspheres from each batch of the formulations ranged from 92.0 to 98.9% (Table 2). This shows that the solvent extraction method is suitable for the formulation of microspheres. It is also an indication that the polymers can form microspheres with the API.

The entrapment efficiency (EE) of the microsphere formulations is shown in Table 2. High values of 76.01 to 80.83% were obtained from the formulations. This indicates that the loaded API is efficiently embedded in the microspheres. EE also increased with polymer concentration, showing that more drug particles are entrapped as the polymer molecules in the formulation increase. There was no significant difference between the EE of the polymers.

The particle size of the polymers (Table 3) was significantly higher than that of the microspheres. A particle size range of between 72 and 84 µm was recorded for all the microsphere formulations (Table 2). This indicates a reduction in particle size of the polymers after being formulated as microspheres which could be due to the increase in surface area of the materials. Increased surface area is a major requirement for mucosal surface adhesion.[29] The marked reduction in particle sizes of the formulation indicates an increase in area-to-volume ratios of the particles; hence the rate of release of the drug from microsphere formulations will also increase. Furthermore, water absorption into smaller particles will be faster because of the shorter distance between the surface and center of the particles; hence there will be an increased rate of swelling. The size and distribution was adequate for optimum absorption across the mucosal layer, the stirring and speed employed in the formulation process probably accounted for the narrow range of particle sizes.[17]

Representative images from the morphological studies of the polymers and microspheres by Scanning Electron Microscopy are presented in Fig. 4d–e. The microspheres were almost spherical with some aggregations. The aggregation between spheres could be due to the adhesive property of the gums. Less aggregation was observed in formulations containing enterolobium gum and HPMC. The microspheres became more spherical with increasing polymer concentration; this is attributable to the polysaccharide composition and gelling properties of the polymers.

Low moisture loss of 3.88–10.24% was obtained in all the formulations (Table 3). This showed that no significant quantity of water was present in the microspheres after formulation.

Table 3. Particle size and size distribution of polymers

Polymer	Particle size (µm)			
	D_{10}	D_{50}	D_{90}	span
Cedrela gum	138.30	436.00	641.40	1.15
Enterolobium gum	120.90	274.70	530.50	1.49
HPMC	130.00	145.00	510.20	2.62

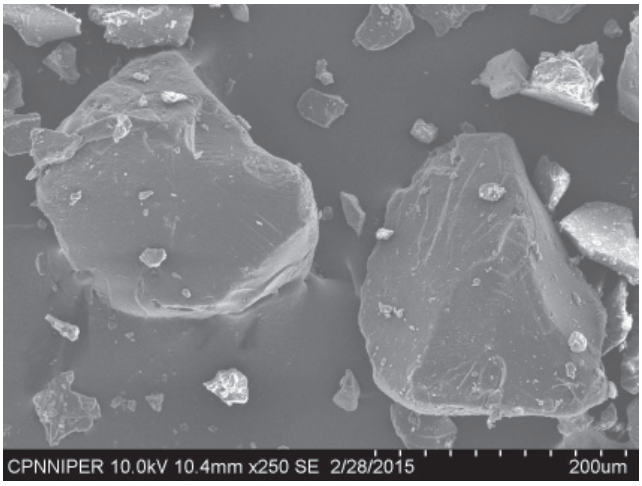

Fig. 4a. SEM images of cedrela gum

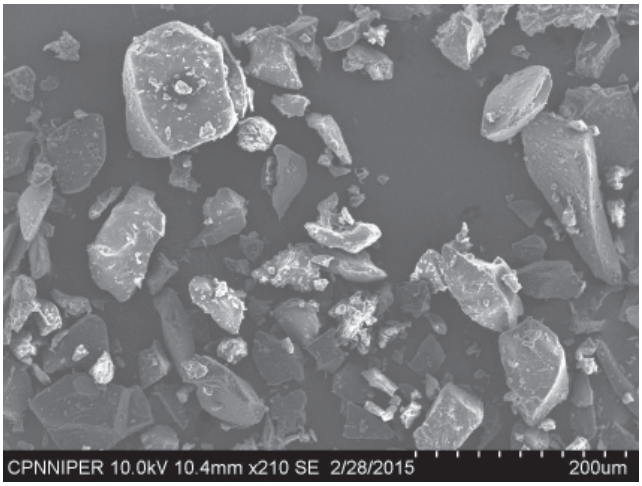

Fig. 4b. SEM images of enterolobium gum

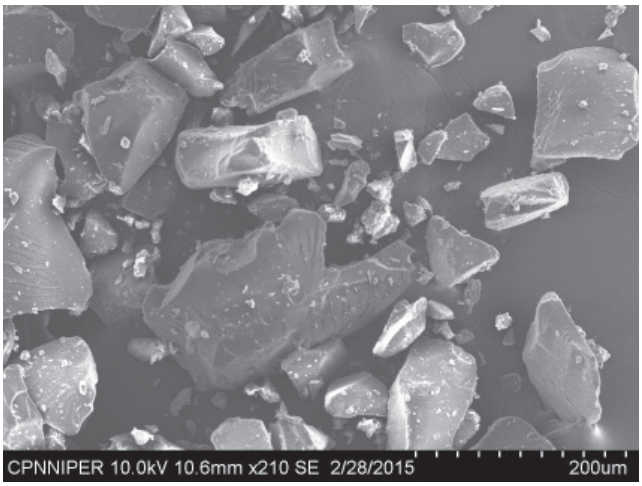

Fig. 4c. SEM images of HPMC

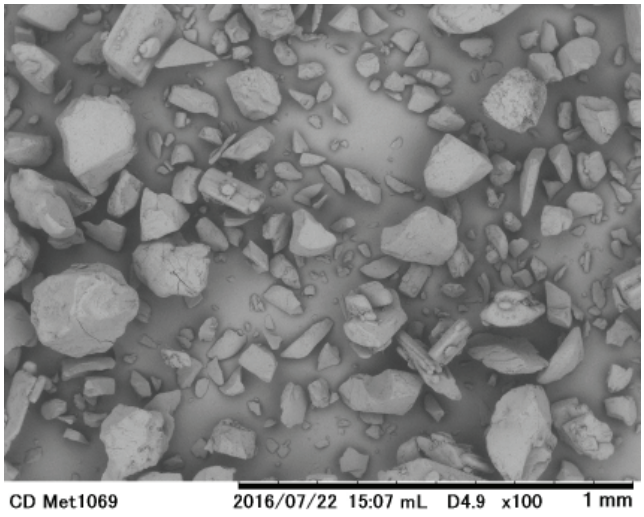

Fig. 4d. SEM images of FC

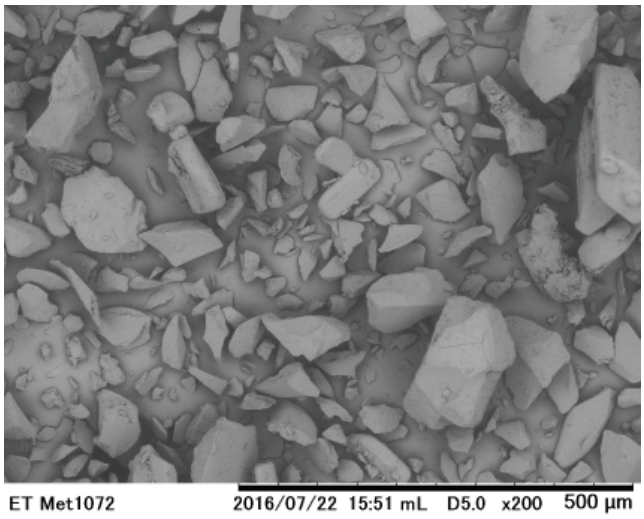

Fig. 4e. SEM images of FE

Release of the drug from microspheres

There was an initial burst release within the 30 min. Thereafter, a constant drug release was observed over 5 h, followed by a much slower release up to day 5 of the dissolution experiment (Fig. 5a–c). Formulations containing enterolobium gum exhibited the highest percentage of drug release, the ranking order was FE > FH > FC, with no significant difference. Furthermore, the mean dissolution time was highest for FE, with a similar ranking order. This indicates that formulations containing cedrela and enterolobium gums are capable of retaining the loaded API for the same period as with HPMC.

To study the mechanism of metformin release from the microspheres, drug release data was fitted into various mathematical models (zero order kinetic, first order kinetic, Higuchi model, Hixson-Crowell and Korsemeyer-Peppas equations) to obtain the coefficient of correlation (r^2) and n values. The values obtained are presented in Table 4.

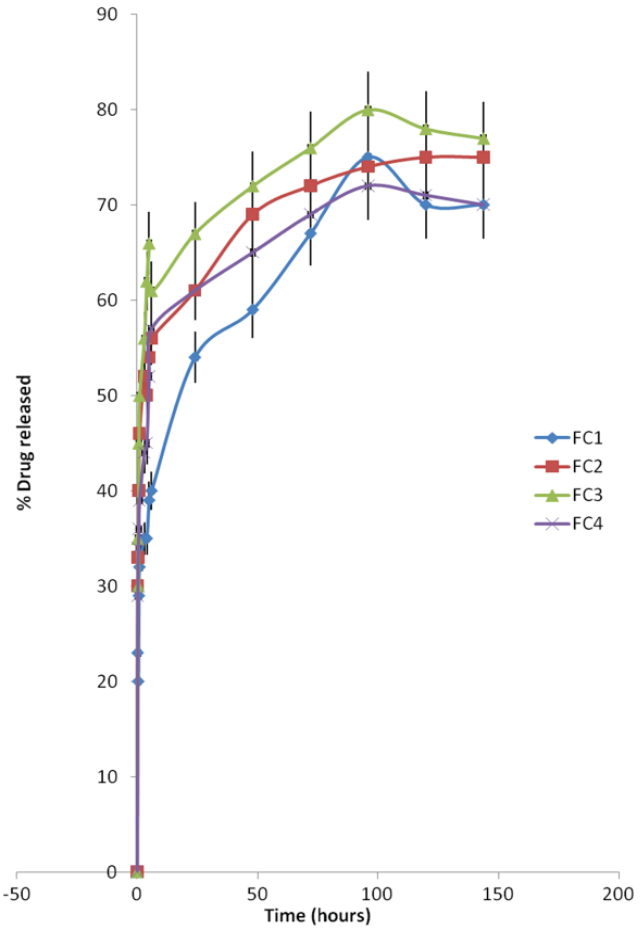

Fig. 5a. Drug release profile of microsphere formulations containing cedrela gum

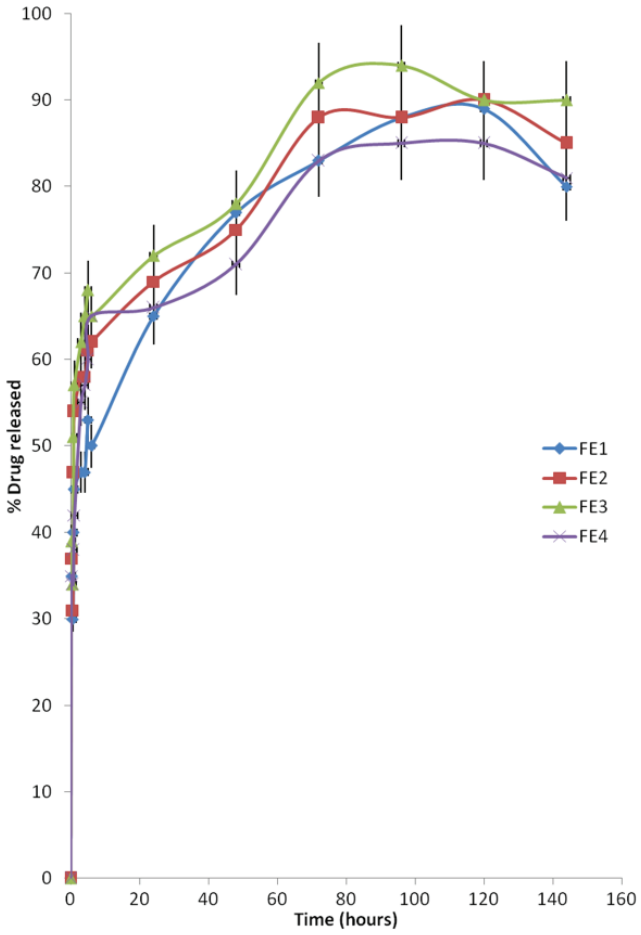

Fig. 5b. Drug release profile of microsphere formulations containing enterolobium gum

The values of n from the Korsmeyer-Peppas model, which is capable of describing the mechanism of drug release from polymeric systems,were considered. [25] When n is 0.43 or less, it indicates release is by diffusion mechanism. When n is 0.85, the mechanism of release is swelling controlled and when n is between 0.43 and 0.85, the mechanism of drug release is by both diffusion and swelling controlled mechanisms; this is termed anomalous.[30] In all the formulations, the values of n were less than 0.43; according to the data obtained, the main mechanism of metformin release from the microspheres is diffusion. Furthermore, all the formulations except FC3 and FC4 had the highest values of r^2 in the Korsemeyer-Peppas equation, while FC3 and FC4 had the highest r^2 values in first order kinetic. This shows that the release of metformin in all formulations except FC3 and FC4 was not concentration dependent while that of FC3 and FC4 depends on the concentration of the polymers. The model-independent dissolution parameters are presented in Table 5. From these parameters, the Mean Dissolution Time (MDT) for the formulations was obtained. The data showed that the formulations were able to sustain metformin for a period ranging from 43 to 46 h, indicating the possibility of reducing the dosing frequency of metformin.

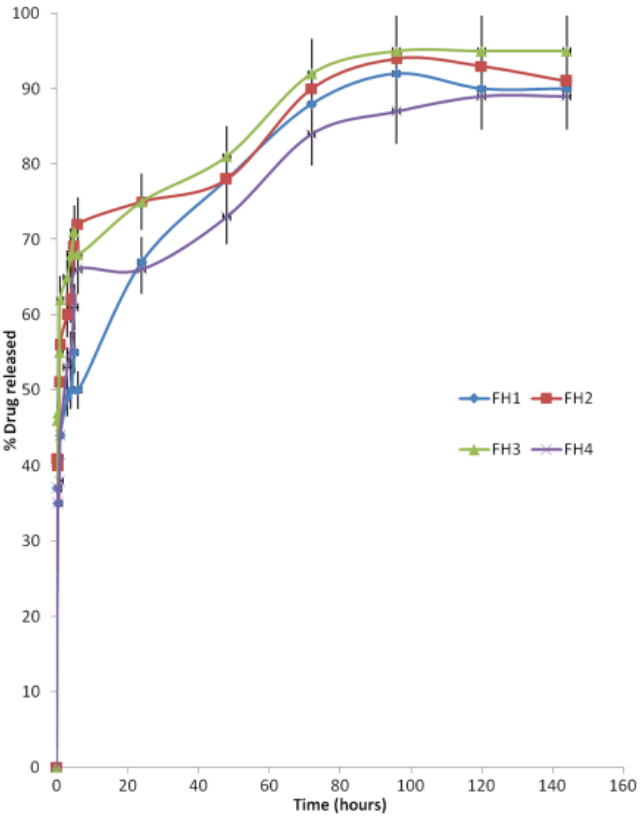

Fig. 5c. Drug release profile of microsphere formulations containing HPMC

Table 4. In vitro release kinetics of microsphere formulations

Formulation code	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsemeyer-Peppas		
	r^2	k_0	r^2	k_1	r^2	k_H	r^2	k_{HC}	N	r^2	k
FC1	0.895	0.671	0.962	0.019	0.962	7.577	0.948	0.004	0.186	0.988	29.53
FC2	0.833	0.719	0.956	0.284	0.913	8.351	0.913	0.009	0.120	0.973	42.84
FC3	0.745	0.755	0.958	0.392	0.836	8.870	0.835	0.010	0.107	0.924	48.04
FC4	0.821	0.684	0.981	0.244	0.910	7.950	0.916	0.008	0.125	0.975	39.97
FE1	0.879	0.813	0.926	0.250	0.952	9.294	0.968	0.010	0.155	0.981	41.06
FE2	0.839	0.839	0.914	0.389	0.912	9.704	0.912	0.010	0.127	0.961	48.21
FE3	0.820	0.875	0.905	0.509	0.895	10.158	0.893	0.010	0.119	0.951	52.12
FE4	0.823	0.799	0.956	0.341	0.903	9.248	0.901	0.010	0.131	0.961	45.35
FH1	0.912	0.859	0.913	0.270	0.973	9.766	0.981	0.010	0.159	0.991	42.48
FH2	0.839	0.884	0.918	0.526	0.913	10.266	0.906	0.010	0.116	0.968	53.39
FH3	0.875	0.907	0.855	0.754	0.937	10.548	0.925	0.011	0.104	0.976	57.40
FH4	0.871	0.837	0.940	0.341	0.935	9.601	0.924	0.010	0.139	0.974	45.40

Mucoadhesive properties

Mucoadhesion is a measure of the strength of contact between the drug delivery system and mucosal surface. The formulations exhibited good mucoadhesion characteristics in this ranking order: FE > FH > FC, with no significant difference (Table 3). There was an increase in peak detachment force with the increasing concentration of polymer in all the formulations, which agrees with the previous report that an increasing concentration of bioadhesive polymer is capable of increasing the binding potential.[5] The polymers, being hydrophilic, absorb water, swell and these enhance mucoadhesion with the mucosal layer. The swelling led to formation of bonds and a spatial network between the mucous membrane and the adhesive polymer in the microspheres. Also, functional groups such as the carboxyl group, present in the gums, are capable of forming hydrogen bonds with the mucin molecules, leading to mucoadhesion.

Conclusion

A mucoadhesive microsphere drug delivery system of metformin hydrochloride was successfully formulated from native *Enterolobium cyclocarpus* and *Cedrela odorata* plant gums. The mechanism of drug release from the microspheres was diffusion. All the formulations exhibited good mucoadhesion properties and a Mean Dissolution Time (MDT) of 43–65 h, which is suitable for a reduced dosing frequency. These native gums may be considered for intestinal drug delivery.

The obtained results are indicative of the need of non-surgical treatment in the group of patients with a past history of myocardial infarction. Continuation of randomized research on a larger group of patients is necessary to obtain a reliable evaluation of therapy effects on the periodontal status.

Table 5. Dissolution parameters of formulations

Formulation code	t_{25}% (h)	t_{50}% (h)	t_{75}% (h)	t_{90}% (h)	MDT (h)
FC1	0.408	17.029	151.100	403.248	61.23
FC2	0.011	3.635	107.529	493.138	63.26
FC3	0.002	1.451	63.650	348.458	63.11
FC4	0.023	6.000	154.072	663.045	65.40
FE1	0.041	3.551	48.215	155.808	52.51
FE2	0.006	1.332	32.236	135.079	52.65
FE3	0.002	0.706	21.023	96.685	48.90
FE4	0.011	2.109	46.716	188.133	57.51
FH1	0.035	2.791	35.873	113.090	46.35
FH2	0.001	0.568	18.680	89.817	48.63
FH3	0.000	0.266	13.009	74.787	43.07
FH4	0.014	2.001	36.946	137.072	52.38

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